

Reproductive and Nutritional Management on Ovarian Response and Embryo Quality on Rabbit Does

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Rabbit does in modern rabbitries are under intensive reproductive rhythms. Females are high milk producers with high energetic expenses due to the extensive overlap between lactation and gestation. This situation leads to a negative energy balance with a mobilization of body fat especially in primiparous rabbit does. Poor body condition and poor health status severely affect the reproductive features (fertility rate and lifespan of the doe as well as ovarian physiology). This paper reviews some reproductive and nutritional approaches used in the last years to improve the reproductive performance of rabbit females, mainly focusing on the influence on ovarian response and embryo quality and with emphasis on epigenetic modifications in pre-implantation embryos and offspring consequences.

Introduction

Rabbit females show special reproductive features as they do not have a well defined sexual cycle, and ovulation is induced by coitus independently of positive oestrogens feedback (Rebollar et al. 1992). Moreover, rabbits show high sexual receptivity immediately after kindling and, as occurs in cattle, insemination can be performed during lactation being females concurrently pregnant and lactating. This situation is particularly negative for reproduction due to nutritional competition, leading to a negative energy balance (NEB) in these animals (Parigi-Bini et al. 1992). This phenomenon is well known and particularly significant at the first parity (Fortun-Lamothe 2006; Arias-Álvarez et al. 2009a).

In the last 20 years, the productivity of rabbit farms has increased and homogenized principally as a result of improvements in management, artificial insemination (AI) and cycled production (Maertens et al. 1995; Castellini 1996), and genetic selection with the use of prolific genetic strains (Bolet et al. 2004; Castellini 2007). In European farms, selected rabbit does are normally under 42-day semi-intensive rhythms (AI on day 11 post-partum and weaning at 35 days of lactation). At this time point (11 days post-partum), their low body condition and hormonal antagonism severely impair fertility and, a critical drop on conception rate has been reported (Theau-Clément and Roustán 1992; Castellini et al. 2003; Arias-Álvarez et al. 2009a). Besides, economical benefits decrease because parturition-interval increases and reproductive lifespan diminish (Fortun-Lamothe 1998; Fortun-Lamothe 2006; Castellini et al. 2010). For this reason, several oestrous synchronization methods and nutritional strategies have

been used to increase female receptivity and reproductive outcome in rabbits does subjected to high production rhythms. These strategies are especially important in primiparous rabbit does, whose reproductive performance is much lower than in multiparous does (Parigi-Bini et al. 1990), and it is reflected in poor ovarian status (Arias-Álvarez et al. 2009a). In any case, both reproductive and nutritional strategies used before or during pregnancy and post-partum period to improve reproductive rabbit management could affect periconceptional events and early embryo development.

Nowadays, management in rabbit production has to take into consideration the recommendations related to animal welfare, the image of rabbit as a 'natural' food and the sensitivity of consumers to considerations of meat quality (Theau-Clément et al. 2005; González-Mariscal et al. 2007; Castellini et al. 2010). Herein, we exposed some reproductive and nutritional strategies to enhance reproductive performance of rabbit females with special interest on the ovarian physiology and embryo quality and development and with emphasis on epigenetic modifications in pre-implantation embryos and offspring derived from does under these strategies. This approach is scarce referred in the literature in this species.

Reproductive Strategies

Intensive and semi-intensive reproductive rhythms used in rabbit production systems require the use of lactating rabbit does. However, these females show depressed sexual receptivity and fertility during lactation period (Ubilla and Rebollar 1995) and require oestrous synchronization methods to increase their lower fertility. Some strategies use hormones to synchronize oestrus for a systematic breeding system but alternative method to hormones have been developed. They are the biostimulation methods. Finally, modifications in the reproductive rhythm have been also explored.

Hormonal stimulation

Sexual receptivity in rabbit does is aphasic and shows a peculiar trend: it is very high immediately after kindling, then decreases with a non-predictable trend, and finally, another peak appears after weaning (Theau-Clément et al. 1990). In rabbit farms, the most widely used hormone in lactating, non-receptive does is equine chorionic gonadotropin (eCG), but the administration

of this hormone induces progressive loss of reproductive efficiency in successive inseminations (Boiti et al. 1995; Rebollar et al. 2006). However, during the first lactations in multiparous does inseminated using this hormonal synchronization treatment follicle development (Rebollar et al. 2008), oocyte cytoplasmic maturation (Arias-Alvarez et al. 2013a), ovulation rate and embryo development (Theau-Clément et al. 2008; Arias-Alvarez et al. 2013a) are improved. Recent study has shown that eCG did not to modify embryo gene expression patterns related to glucose intake, proliferation and differentiation, apoptotic-induced pathways, capacity of implantation and foetal growth, but embryos from eCG-treated mothers showed a lower antioxidant defence after the maternal eCG treatment (Arias-Alvarez et al. 2013a). Such oxidative events may be implicated in the reduced prolificacy and fertility rates observed in females when eCG administration is used repeated and consecutive times. Further studies are warranted to deepen our knowledge regarding gene expression patterns and oxidative stress when hormonal treatments are used in the reproductive protocols applied in rabbits.

Biostimulation methods

Biostimulation methods are natural and less expensive alternative to hormonal treatment. Some of the methods that have been tried include control lighting, transient doe-litter separation and changing does to another cage (revised by Theau-Clement 2008) among others. Several of the more successful strategies are described below.

Lactation control

As previously has mentioned, lactation negatively influences the reproductive functions in the rabbit by depressing follicle development (Kermabon et al. 1994), oocyte quality (García-García et al. 2012), ovulation rate, fertilization and embryo development (For-

tun-Lamothe and Bolet 1995). Also, sexual receptivity and fertility are low during the period of lactation (Ubilla and Rebollar 1995). For this reason, lactation control appears a very effective method to stimulate ovarian function previous AI.

Doe-litter separation on specific days of lactation before insemination was as effective as eCG treatment, especially for the first four inseminations applied at 4 days post-partum (Rebollar et al. 2006). It results in a decrease in plasma prolactin concentrations that could promote growth of follicular waves and high steroidogenesis activity (Ubilla et al. 2000; Rebollar et al. 2008). It can be performed for 24–48 h in early lactation (Bonanno et al. 2002) or nursing with a short controlled suckling in a 48-h period of doe-litter separation (Bonanno et al. 2004). Another method is applying a 2-day controlled nursing before insemination, by allowing for a 10-min nursing of the litter at 24 h of separation (Rebollar et al. 2008). In all strategies, receptivity and fertility rates are improved.

Transient doe-litter separation for 24 h can be also applied; it improves ovarian follicle and oocyte quality compared with an eCG treatment in primiparous does at Day 11 post-partum (Arias-Alvarez et al. 2010a; Fig. 1). Biostimulated does showed lower number of mid-atretic follicles and highly cytoplasmic maturation rate of oocytes, in terms of cortical granule migration rate (Fig. 2) compared to does synchronized with eCG (Arias-Alvarez et al. 2010a,b). Therefore, different treatments should be used along the reproductive life of the does taking into account their reproductive rhythm and age.

In commercial rabbit farms, litter weaning usually occurs from Days 28 to 35 of age. Another method for lactation control tried has been early weaning as a strategy to decrease energy deficit of primiparous lactating rabbit does prior to insemination in a more extensive rhythm. It can decrease the energy deficit by reducing the lactation duration (Xiccato et al. 2004) and

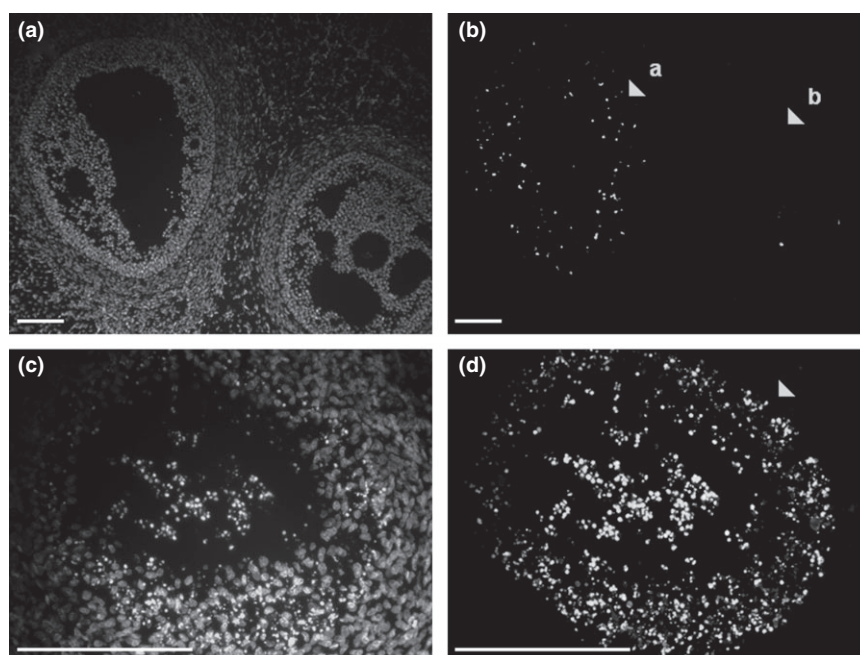
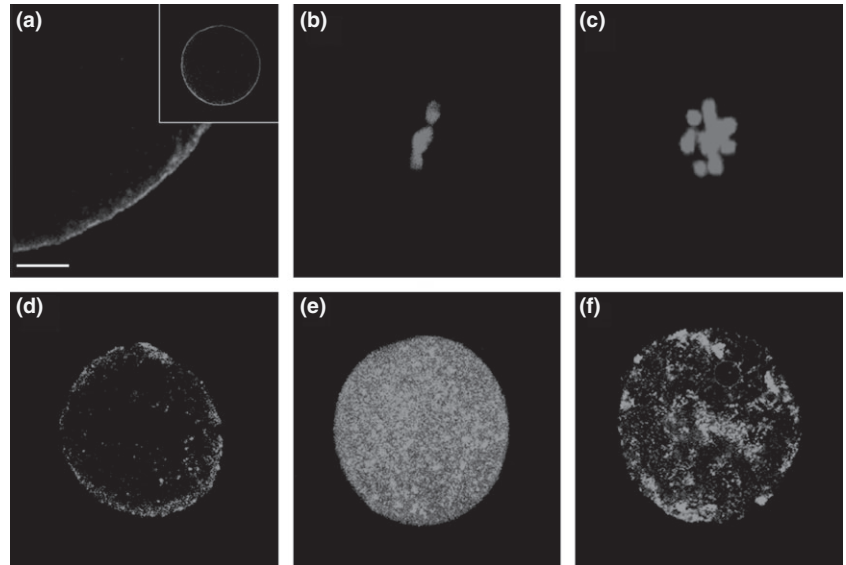


Fig. 1. Follicular apoptosis measured by the TUNEL technique in ovarian sections of primiparous rabbit does. (A,B) Control sections showing ovarian follicular cells marked with DAPI; (C) Follicular apoptotic cells are labelled, showing an early atretic follicle (a, arrow) and a non-atretic follicle (b, arrow); (D) Follicular apoptotic cells are labelled, showing a late atretic follicle (arrow). Scale bar = 200 μ m (from Arias-Alvarez et al. 2010a,b)

Fig. 2. Nuclear and cytoplasmic oocyte maturation in terms of metaphase II and cortical granule (CG) migration in primiparous rabbit does treated with eCG or biostimulated: (A) Peripheral CG distribution; (B,C) Metaphase II; (D) Cortical CG distribution; (E) Homogeneous CG distribution; (F) Non-homogeneous or abnormal CG distribution. Scale bar = 10 μ m. Oocyte diameter is approximately 80 μ m (from Arias-Álvarez et al. 2010a,b)



improving body reserves (Feugier and Fortun-Lamothe 2006; Sakr et al. 2010), but this strategy did not give rise to improve ovarian status in terms of follicle development or oocyte *in vitro* maturation rates (Sakr et al. 2010), number of corpora lutea (Xiccato et al. 2005) and fertility rate (Feugier and Fortun-Lamothe 2006). Moreover, kit mortality increases 3.6-fold in early weaning at 25 days compared with weaning performed at 35 days of age (Rebollar et al. 2009).

Reproductive rhythm

Reproductive performance seems to be affected in continuous intensive rhythms compared with semi-intensive ones (Cervera et al. 1993; Rebollar et al. 2006; García-García et al. 2009). It has been reported that an extensive reproductive rhythm can be adapted to enhance rabbit nutritional status, fertility, length of reproductive activity and welfare (Castellini et al. 2003, 2010) as they eliminate the hormonal and energetic antagonisms between lactation and pregnancy (Castellini et al. 2003). Our own studies have demonstrated that insemination after weaning is the best management, at least for primiparous does (Arias-Álvarez et al. 2009a; Rebollar et al. 2009). It improves estimated body composition and energy content, the serum leptin and protein concentrations, the health of follicular populations in the ovary, showing lower apoptosis rate

and the oocyte quality, by enhancing oocyte maturation compared with semi-intensive rhythm (Fig. 3). Conception rate and prolificacy are substantially improved as a consequence (Arias-Álvarez et al. 2009a).

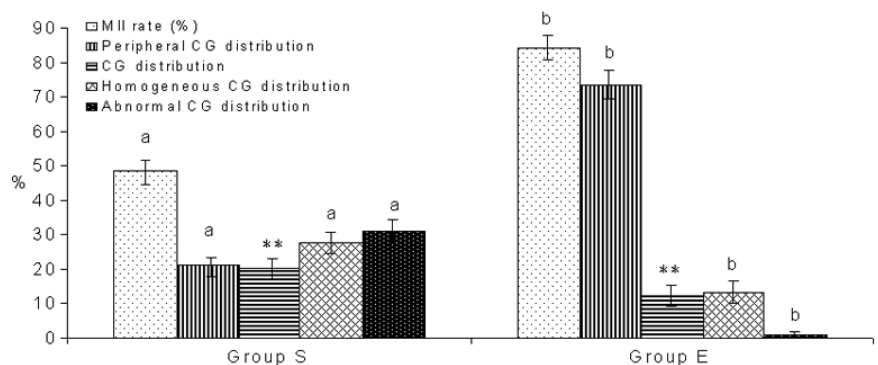
Nutritional Strategies

Energy deficit during first lactation lead to the mobilization of body reserves (Xiccato et al. 1999) and shifts in blood metabolites. These changes are reflected in the environment of the growing and maturing female gamete that compromise oocyte competence and embryo survival and fertility (Castellini et al. 2006; Arias-Álvarez et al. 2009a). However, the relationship between maternal nutritional status and the earliest events of reproductive function such as folliculogenesis, oocyte maturation, pre-implantation embryo development, uterine environment and the secretion of progesterone has been poorly studied in rabbit. Then, we describe some of the nutritional management approaches tried to increase diet energy level before insemination.

Rearing diets

In rearing period, some strategies have been studied to improve nutritional statement of subsequent primiparous females. Overfeeding or *ad libitum* diets in young

Fig. 3. *In vitro* oocyte maturation in primiparous does inseminated under semi-intensive or extensive rhythm. Nuclear and cytoplasmic maturation was measured in terms of metaphase II rate (MII) and cortical granules migration. Different letters in the same column (a,b) and (c,d) indicate significant differences ($p < 0.001$) and ($p < 0.05$), respectively. ** ($p = 0.08$) (from Arias-Álvarez et al. 2009a)



females before first mating are inadequate because it can lead to fattiness status and subsequently poor fertility results (Castellini et al. 2010; Martínez-Paredes et al. 2012; Szendro et al. 2012). Feed restriction or earlier use of suitably fibrous diets is preferred to lead females to achieve the critical body weight and fat mass at first mating to ensure reproduction (Martínez-Paredes et al. 2012).

In last years, many authors agree on the importance of specific feeding plans, especially with fibre-rich diets for young does (Xiccato et al. 1999; Rommers et al. 2004). High-lignin, fibre-rich diets administered to nulliparous rabbit does improved voluntary feed intake in females during pregnancy (García et al. 1999; Nicodemus et al. 1999; Arias-Álvarez et al. 2010b; Rebollar et al. 2011), and consequently, energy ingestion and metabolic status in the early lactating period when a more energetic feed is furnished (Xiccato et al. 1999; Pascual et al. 2003). Some works even reported enhanced conception rate (Xiccato et al. 1999; Pascual et al. 2003). However, energy reserves and some endocrine parameters as serum leptin levels or estradiol seems to be lower at first insemination (Fig. 4), and it is reflected in lower *in vitro* embryo survival rate and conception rate in another study (Arias-Álvarez et al. 2009b). Recently, it has recommended the introduction of a short flushing around first AI when fibrous diets are used because good fertility rates at the first parturition in nulliparous rabbits are obtained (Martínez-Paredes et al. 2012).

Although feed intake increased during rearing and first pregnancy in does fed with a high-lignin-based diet during the rearing period, unfortunately it did not sufficiently enhance feed intake during first lactation, metabolic status or body composition neither reproductive status. These animals showed higher rate of follicular apoptosis, lower index of oocyte *in vitro* maturation and lower number of *in vivo*-recovered viable embryos compared with those fed with a standard lignin diet at Day 11 post-partum (Arias-Álvarez et al. 2010b).

Energy supplementation

Another approach used to improve NEB during lactation is the energy supplementation with glycolic supplements as propylene glycol (PG) to increase plasma glucose and insulin to improve the metabolic and endocrine female status. Increased folliculogenesis and ovulation rates have been demonstrated with the administration of energy yielding compounds in other species (cow: Miyoshi et al. 2001; ewe: Letelier et al. 2008), but controversial results are found (cows: Moallem et al. 2007; Rizos et al. 2008). In rabbits, PG seems to have positive effects on sexual receptivity and fertility, but these results are not conclusive (Luzi et al. 2001). Short-term supplementation (flushing 4 days before insemination) of PG in rabbits did not improve metabolic reserves, follicular development, oocyte quality or reproductive performance in primiparous lactating does (Sakr 2012).

In addition, maternal supplementation with PG from mid-pregnancy and during lactation (long-term flushing) was not able to modify the rabbit metabolic status (serum glucose, insulin and NEFA concentrations). Ovarian response in terms of ovulation rate, follicular apoptosis, oocyte maturation and number of blastocysts developed were also not improved with this long-term flushing (Arias-Álvarez et al. 2013b). However, embryos from mothers supplemented with PG over a long term seems to adapt their 'own metabolic pathways' as they showed higher mRNA transcripts involved in glucose metabolism, proliferation and differentiation, implantation capacity and foetal growth (*SLC2A4*, *INSR*, *IGF1R*, *PLAC8*, *COX2* and *IGF2R*) without an increase in oxidative stress and associated apoptotic pathways (*SOD1*, *NOS3* and *TP53*) (Arias-Álvarez et al. 2013b) (Fig. 5). This may represent an accurate regulation in redox balance in rabbit blastocysts and a potential chance for embryo survival and successful implantation in those from females supplemented with PG (El-Sayed et al. 2006).

It is known that the increase of the digestible energy of the diets to improve the NEB during lactation using fat

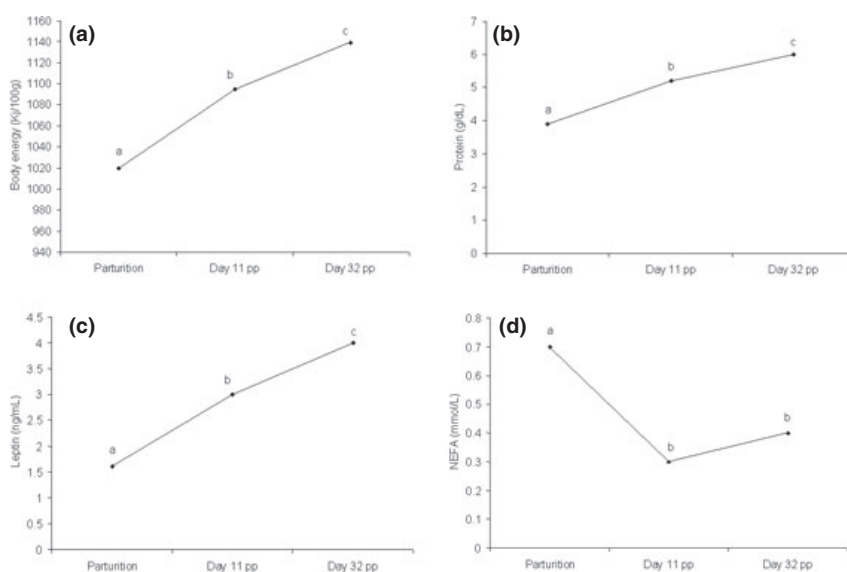


Fig. 4. Body energy content (A) and serum parameters related to metabolic status (B–D) measured in different time points along *post-partum* period [at parturition, on day 11 *pp* (lactation) and on 32 *pp* (post-weaned)] in primiparous rabbit does. All samples were recovered at 9:00 a.m. Different letters in the same column (a–c) and (d–f) indicate significant differences ($p < 0.001$) and ($p < 0.05$), respectively (from Arias-Álvarez et al. 2009b)

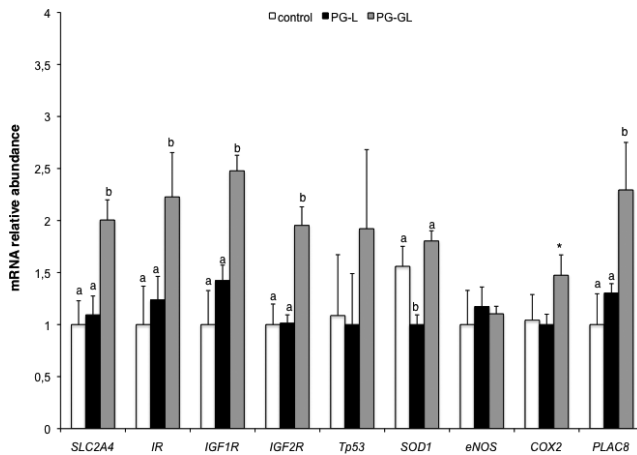


Fig. 5. Relative abundance of *SLC2A4*, *IR*, *IGF1R*, *IGF2R*, *Tp53*, *SOD1*, *eNOS*, *COX2* and *PLAC8* mRNA in blastocysts recovered (Day 28 *post-partum*) from does supplemented with 2.5% of propylene glycol during gestation and lactation (PG-GL), during lactation (PG-L) or not supplemented (Control). Error bars represent SEM. Means in bars with different letters differ significantly ($p < 0.05$), * $p = 0.09$ (from Arias-Álvarez et al. 2013b)

or/and starch seems ineffective in rabbits because the higher energy intake was accompanied by a simultaneous increase in milk production (Fortun-Lamothe 1998). High-fat diets seem to improve the productivity of lactating does and their corporal condition during the first lactation (Pascual et al. 1998). However, high cholesterol diet administered from before puberty onwards affected onset of puberty, follicular growth and embryo gene expression in the very early stages of pregnancy, as well as hormonal responses to breeding and GnRH stimulation in relation to age leading to foetal induced intra-uterine growth retardation (Picone et al. 2011; Cordier et al. 2013). High-starch diet increased the live weight and the energy intake of primiparous rabbit does during lactation, but it did not improve the performance of their litters and could induce an excessive live weight of gestating rabbit does (Pascual et al. 1999).

Other supplements or strategies

Last times, dietary supplementation with fish meal or omega-3 fatty acids has studied in lactating cow (Heravi Moussavi et al. 2007; Silvestre et al. 2011). Altering the n-3 PUFA content in rabbit tissues by dietary intervention has been proving effective and has important implications related to animal and consumers health (Harris, 2007). Because they influence reproductive physiology as they are implicated in the prostaglandin and cholesterol metabolism; it could reduce $\text{PGF}_{2\alpha}$ secretion, which would increase fertility and reduce pregnancy losses. In rabbits, nulliparous females fed 1.5% concentrated rich in omega-3 fatty acids improved

serum progesterone concentration around embryo implantation time and docosapentaenoic acid, eicosapentaenoic acid, and docosahexaenoic acid concentrations in periovarian fat (Rebollar et al. 2014). Long-term supplementation with this diet did not affect endocrine and metabolic parameters in rabbit does, but long-term effect on the number, weight and size of stillborn after second parturition has been suggested.

Fasting

Nutritional status of the does, as modified by fasting, greatly influenced fertility. Before ovulation, a group of hormones and oxidizable metabolites are involved in signalling the energy status of the rabbits to the central reproductive axis and ovary, thus influencing follicular development and oocyte quality. Fasting at pre-conception time affects fertility rates and sexual receptivity rather than the ovulatory index in rabbits (Brecchia et al. 2006). Severe fasting for 48 h affected serum metabolic signals to the reproductive system like NEFA and leptin concentrations, ovarian endocrine parameters, such as E2 levels in the luteal phase, and embryo development and embryo developmental speed. However, embryonic gene expression of some candidate genes implicated in metabolism, apoptosis and embryo development remained almost unaltered independent of the acute food deprivation (García-García et al. 2011a,b).

Conclusion

The combination of some reproductive and nutritional strategies for better management of energy balance, improvement of the body status and then reproductive outcome is required, as such as a multi-factorial approach. These strategies must be adapted to rabbit doe physiological ovarian response and take account the epigenetic changes that can be occasioned in embryos and subsequent offspring.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All authors have made equal contributions to this publication (designed study, analysed data and drafted paper).

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